



# Thermodynamics of binding of cadmium to bovine serum albumin

Song-Sheng Qu, Yi Liu \*, Tian-Zhi Wang, Wen-Ying Gao

Department of Chemistry, College of Chemistry and Molecular Science, Wuhan University, Wuhan 430072, People's Republic of China

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## Abstract

The binding isotherm of  $\text{Cd}^{2+}$  ion to bovine serum albumin (BSA) has been investigated by microcalorimetry at 310.15 K and pH 7.0. The thermodynamic parameters of the binding reaction have been determined, and the stoichiometry of the complex is 2:1, indicating that there exist two identical binding sites of BSA with  $\text{Cd}^{2+}$  ion. The value of  $\Delta_r H_m^\ominus$  is  $-28.4 \pm 1.7 \text{ kJ mol}^{-1}$ , the free energy of binding  $\Delta_r G_m^\ominus$  is  $-25.2 \text{ kJ mol}^{-1}$ , and the entropy of binding  $\Delta_r S_m^\ominus$  is  $-10.3 \text{ J mol}^{-1} \text{ K}^{-1}$ . The negative  $\Delta_r H_m^\ominus$  and  $\Delta_r S_m^\ominus$  values are observed for the binding reaction of  $\text{Cd}^{2+}$  ion and BSA, suggesting that the binding reaction is mainly enthalpy-driven and the entropy is unfavorable for it. © 2002 Elsevier Science Ltd. All rights reserved.

**Keywords:** Microcalorimetry; Bovine serum albumin;  $\text{Cd}^{2+}$  ion; Thermodynamics

## 1. Introduction

The serum albumins are the most abundant protein in blood serum, which belong to a multigene family of proteins that includes  $\alpha$ -fetoprotein and human group-specific component or vitamin D-binding protein (He and Carter, 1992). They have a single polypeptide chain of amino acids with a largely-helical triple-domain structure involved in the binding, transport and delivery of a range of endogenous small molecules, as well as drugs and xenobiotic (Kragh-Hansen, 1981; Squella, 1987; Ogata, 1990). Serum albumins have been also implicated in transport, storage, and metabolism of many metal ions, and have many physiological functions as the major constituents of the circulatory system (Laurie and Prtt, 1986; Masuoka et al., 1993; Masuoka and Saltman, 1994; Sadler and Viles, 1996; Wang, 1998). The characterization of metal ions

binding to serum albumin is vital to our understanding of the relationship between structure and function. A fundamental parameter of metal binding activity is the intrinsic binding constant, which describes that the affinity with metal ions is bound to specific serum albumin sites.

Calorimetry has contributed a great deal to our current understanding of the mechanisms of regulation and control of biological structures and processes at the molecular level. Its advantage is that the method directly measures the “heat signal” occurrence of the binding process and thereby avoids the need to partition between the free and bound ligands. It has been used widely in the determination of thermodynamic parameters. In fact, all biological processes depend critically on the binding of ligands, by specific protein. Because nearly all binding interactions are accompanied by a change in enthalpy, and all reactions of interest will produce a calorimetric signal, the calorimetry offers the possibility of directly determining not only the binding constant, and thereby free energy, but also the stoichiometry, enthalpy, and entropy in a single experiment (Gilli et al., 1998; Keown et al., 1998).

\* Corresponding author. Tel.: +86-27-872-18284; fax: +86-27-876-47617.

E-mail address: liuyi@chem.whu.edu.cn (Y. Liu).

$\text{Cd}^{2+}$  ion being a common toxic element, about 90% of it in blood is bound to serum albumin and  $\alpha_2$ -macroglobulin (Scott and Bradwell, 1983) and 50% of this is bound tightly to  $\alpha_2$ -macroglobulin and is non-exchangeable (Watkins et al., 1977), but the rest is bound to albumin and is readily exchangeable (Giroux and Henkin, 1972). Both the biological functions and the toxicities of  $\text{Cd}^{2+}$  ion have drawn people's attention. Many studies on the interaction and structure of  $\text{Cd}^{2+}$  ion to serum albumin have been reported (Suzuki et al., 1986; Zhou et al., 1992). However, no experimental data on the enthalpy and entropy of the binding of  $\text{Cd}^{2+}$  ion to BSA were reported. The thermodynamic parameters can provide fundamental information of structure and of albumin. To gain a quantitative assessment of these parameters, we have employed microcalorimetry for determining the heat changes that accompany  $\text{Cd}^{2+}$  ion binding to BSA. Besides, the binding constant and stoichiometry, values for  $\Delta_r H_m^\ominus$  and  $\Delta_r S_m^\ominus$  can also be calculated from the calorimetric experiments. With these data in hand, the nature of  $\text{Cd}^{2+}$  ion binding to BSA can be more rigorously analyzed. In this paper, we have therefore studied the binding of  $\text{Cd}^{2+}$  to BSA by microcalorimetric method, and provided a thermodynamic description based on the complex reaction.

## 2. Experimental

### 2.1. Materials

BSA, being electrophoresis grade reagents, was purchased from Huamei Biological Engineering. The buffer Tris had a purity of no less than 99.5% and  $\text{CdCl}_2$ , NaCl, HCl, EDTA, etc. were all of analytical purity. All solutions were used with doubly distilled water. There was  $0.05 \text{ mol dm}^{-3}$  NaCl in the protein solutions, and  $0.05 \text{ mol dm}^{-3}$  Tris-HCl buffer was used to maintain their  $\text{pH} = 7.0$ . The solutions of albumin were freshly prepared, and the concentration determined spectrophotometrically (Edwards et al., 1969). The concentration of the  $\text{CdCl}_2$  solution was determined by titration with EDTA, then diluted to be suitable for the calorimetric experiments. The effect of  $\text{Cl}^-$  ion can be eliminated with buffer of Tris-HCl, which contains large amounts of  $\text{Cl}^-$  ion.

### 2.2. Microcalorimetry

Calorimetric experiments were carried out at 310.15 K using an LKB-2107 batch microcalorimeter system. One of the main components of the instrument consists of two separate calorimeter cells (Fig. 1), one of which is the reaction cell and the other is the reference cell, each cell being divided into two parts.

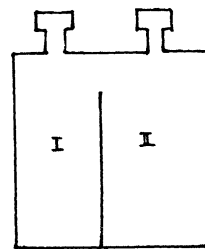


Fig. 1. The calorimeter cell.

$4.00 \text{ cm}^3$  of the BSA solution and  $2.00 \text{ cm}^3$  of  $\text{Cd}^{2+}$  solutions, already separately diluted to the required concentration by buffer solution, were placed in the compartments II and I of the reaction cell, respectively. Consequently, the final BSA concentrations were equal to two-third of the initial concentrations. In order to avoid the influence of the heat of mixing on the results of the measurement, the contents and quantities in both cells were made as identical as possible except BSA was not added to compartment I of the reference cell. When the microcalorimetry system had been equilibrated and a steady baseline obtained on the recorder, the reaction run was initiated by starting rotation of the calorimeter so as to mix the BSA and  $\text{Cd}^{2+}$  solutions. Consequently, the heat generated in the reaction process was recorded by means of LKB-2210 dual-pen integrating recorder. Introducing a known quantity of electric power into the electric calibration heater carried out the calorimetric calibration.

### 2.3. Calculation of the thermodynamic parameters

The heat of  $\text{Cd}^{2+}$  ion binding to BSA protein was measured as a function of  $\text{Cd}^{2+}$  ion concentration ( $L$ ). The experimental heat effect ( $Q_e$ ) associated with mixing of the reactants is related to the heat of binding ( $Q_b$ ) and the heat of dilution of the protein ( $Q_{\text{dil}}$ )

$$Q_e = Q_b + Q_{\text{dil}}. \quad (1)$$

The heat of dilution of the protein can be instrumentally determined. After subtraction of the heat of dilution, the heat of binding is proportional to the quantity of  $\text{Cd}^{2+}$  ion and protein complex formed with the protein concentration fixed as  $P$ , as follows (Freire et al., 1990):

$$Q_b = V \cdot \Delta_r H_m^\ominus \cdot L_b, \quad (2)$$

where  $\Delta_r H_m^\ominus$  is the binding enthalpy per mole  $\text{Cd}^{2+}$  ion,  $L_b$  is a bound concentration of  $\text{Cd}^{2+}$  ion and  $V$  is the total volume of solution equal to  $6.00 \text{ ml}$ . For a system containing multiple sets of independent binding sites, the concentration of metal ion bound ( $L_b$ ) is given by (Freire et al., 1990)

$$L_b = P \sum_{m=1}^i \frac{n_i K_i L_f}{1 + K_i L_f}, \quad (3)$$

where  $m$  is the number of classes of independent binding sites such that each class ( $i$ ) has  $n_i$  sites with the binding constant ( $K_i$ ), and the free metal ion concentration ( $L_f$ ) can be expressed by the mass conservation expression in the following equation:

$$L = L_f + L_b. \quad (4)$$

For the simplest single-class binding model ( $m = 1$ ), the Eq. (3) becomes

$$Q_b = \frac{V \cdot \Delta_r H_m^\ominus (A - \sqrt{A^2 - 4n_1 \cdot P \cdot L})}{2}, \quad (5)$$

where

$$A = 1/K_1 + n_1 P + L.$$

The binding and thermodynamic parameters,  $K_1$ ,  $N_1$  and  $\Delta_r H_m^\ominus$  can be computed from actual calorimetric data by a non-linear fitting carried out with Micro cal Origin Software using Eq. (5).

The binding constant  $K$  is related to the free energy  $\Delta_r G_m^\ominus$  by the well-known relation

$$\Delta_r G_m^\ominus = -RT \ln K, \quad (6)$$

where  $R$  is the gas constant and  $T$  is the temperature in K. The free energy is again composed of a heat term ( $\Delta_r H_m^\ominus$ ) and an entropy ( $\Delta_r S_m^\ominus$ ), related by another fundamental equation

$$\Delta_r G_m^\ominus = \Delta_r H_m^\ominus - T \Delta_r S_m^\ominus. \quad (7)$$

### 3. Results and discussion

Calorimetric experiments of BSA with  $\text{Cd}^{2+}$  ion were performed at 310.15 K, pH = 7.0. Example of the heat changes accompanying the binding of incremental additions of  $\text{Cd}^{2+}$  ion to BSA is shown in Fig. 2. Analysis of the processed calorimetric curve (Fig. 2) in terms of a simple single-class binding model shown that binding is exothermic. The curve was obtained by using Eq. (5) and lead to the following best-fit values:  $n = 2.1 \pm 0.1$ ,  $K = (1.76 \pm 0.12) \times 10^4 \text{ mol dm}^{-3}$  and  $\Delta_r H_m^\ominus = -28.4 \pm 1.7 \text{ kJ mol}^{-1}$ . The free energy of binding ( $\Delta_r G_m^\ominus = -25.2 \text{ kJ mol}^{-1}$ ) and the entropy of binding ( $\Delta_r S_m^\ominus = -10.3 \text{ J mol}^{-1} \text{ K}^{-1}$ ) were calculated from Eqs. (6) and (7), respectively.

The negative  $\Delta_r H_m^\ominus$  and  $\Delta_r S_m^\ominus$  values of the binding reaction of  $\text{Cd}^{2+}$  ion and BSA, indicate that the binding is mainly enthalpy-driven and the entropy is unfavorable for it. The decrease in entropy suggests an increased degree of orderliness on complex, resulting from the

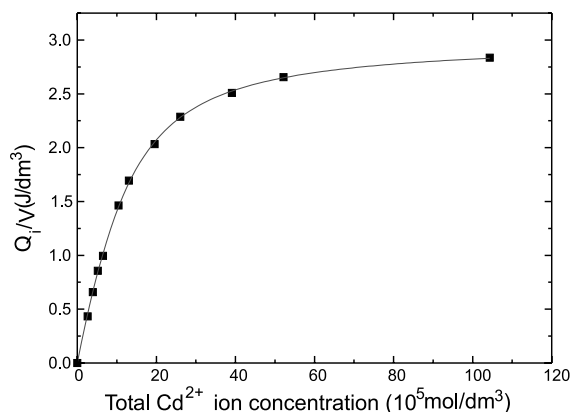


Fig. 2. The calorimetric curve of  $\text{Cd}^{2+}$  ion binding to BSA. The concentration of BSA is  $5 \times 10^{-5} \text{ mol dm}^{-3}$ .

bimolecular  $2 \rightarrow 1$  reaction (Testa et al., 1987). The  $n = 2.1$  indicates that the stoichiometry of  $\text{Cd}^{2+}$  ion binding to BSA is 2:1 complex and the differences between two  $\text{Cd}^{2+}$  ion are not significant in thermodynamic.

The detected binding sites of  $\text{Cd}^{2+}$  ion to BSA are consistent with NMR studies.  $^1\text{H}$  and  $^{113}\text{Cd}$  NMR studies suggested that there are two similar strong  $\text{Cd}^{2+}$  binding sites (Martins and Drakenberg, 1982; Sadler and Viles, 1996), and the two strong sites do not involve the free thiol at cys34 of BSA. Gömüakos et al. (1991) have determined the affinities of equilibrium dialysis. They found there are two strong binding sites, and one much weaker site. In our studies, we failed to detect the weaker binding site which may bind  $\text{Cd}^{2+}$  too weakly to be used in this calorimetric method. The result of two strong binding sites that is determined by equilibrium dialysis is in good accordance with our experimental result, too.

In recent years, isothermal titration calorimetry has been developed and widely used to measure the energetics of biological reactions or molecular interactions (ligand-binding phenomena, enzyme-substrate interactions, and interactions among components of multi-molecular complexes) in a single experiment at constant temperature (Bundle and Sigurskjöld, 1994). It made possible direct thermodynamic characterization of association processes exhibiting very high affinity binding constants ( $10^8$ – $10^9 \text{ mol dm}^{-3}$ ) that are frequently found in biological reactions, having capability of measuring heat effects arising from reactions involving as little as nanomole amounts of reactions (Donner et al., 1982; Schon and Freire, 1989; Freire et al., 1990), and did not require large amounts of protein. Such protein concentrations were not compatible with their solubility or the difficulty in obtaining several milligrams of these expensive macromolecules. Thus, we believe that calorimetry will be widely used in biological reactions.

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